

flies whose hemolymph was examined with phase-contrast microscope for *Treponema* are shown in Table 1. Almost the entire progeny of the *D. bifasciata* females injected with *Treponema*-containing hemolymph of *D. willistoni* were infected, and the infected females have transmitted the parasites to their progenies, at least up to and including the F₄ generation. Also, *D. bifasciata* males withstand the infection better than do *D. willistoni* males; only a minority of the males of the former species die, and the remainder are both viable and fertile.

The question now is whether *Treponema* which has lived for one fly generation or more in *D. bifasciata* preserves its original properties, or whether it becomes in some sense a less virulent strain. This was tested by injecting the hemolymphs of the infected *D. bifasciata* and *D. willistoni* into Oregon-R females of *D. melanogaster*. The progenies of the latter females, produced after 3 to 5 days of incubation, were examined for unisexual, or almost unisexual, progenies. The results are shown in Table 2. The *Treponema*, although

relatively innocuous to *D. bifasciata*, is still able to infect and to produce the usual effect, unisexual progenies, in *D. melanogaster*.

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Phytadienes in Zooplankton

Abstract. Four isomeric phytadienes have been isolated from mixed zooplankton of the Gulf of Maine. The chemical structures suggest that the mixture is derived by dehydration of phytol (presumably by acid catalysis), which is present in the diet of the zooplankton.

Chromatographic analyses of bulk zooplankton extracts from the Gulf of Maine yielded a saturated hydrocarbon fraction consisting predominantly of pristane (2,6,10,14-tetramethylpentadecane) (I, 2) and an unsaturated fraction containing a complex mixture of olefins. We wish to report the struc-

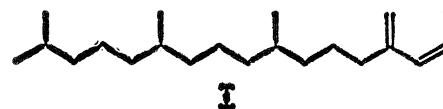
tures of four closely related olefins with gas chromatographic retention indices between 1900 and 2000 (Table 1), measured on a polar column (3.5 percent Carbowax C20M on Chromosorb G, acid washed, treated with dichloromethylsilane).

All four compounds have, by mass

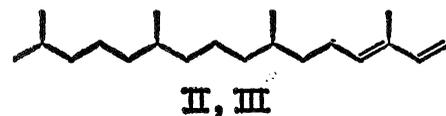
spectrometry, a molecular weight of 278; catalytic hydrogenation of the individual hydrocarbons yields phytane (2,6,10,14-tetramethylhexadecane; molecular weight 282). Phytane was synthesized as a reference compound by hydrogenation of phytadienes that were obtained by catalytic dehydration of phytol (3). The identity of the two products was shown by their retention indices (Table 1) in gas chromatography and by their infrared and mass spectra.

The olefins thus characterized as four isomeric phytadienes were separated by small-scale preparative gas chromatography and were further examined by infrared and ultraviolet spectroscopy and by ozonolysis. Ozonides were prepared from very small (microgram) quantities of the olefins in a thin film free of solvent, at the temperature of dry ice. They were studied by oxidation to the acids, by catalytic or triphenylphosphine reduction to the aldehydes, or—with the best yields—by pyrolysis in the gas chromatographic inlet system at 200°C. The products were identified by their retention indices, and the type of substitution was determined from the infrared spectra of pure samples trapped from the effluent of the column.

The infrared spectrum of compound I shows terminal vinyl and terminal methylene unsaturation, and the ultraviolet absorption indicates a monosubstituted conjugated diene. Ozonization followed by oxidation and esterification produced a methyl ester of a C₁₇ acid. In infrared spectrum and retention index compound I is identical with synthetic neophytadiene (I) (3).



The presence of a trisubstituted ethylene structure conjugated with a terminal vinyl group is indicated by the infrared spectra of compounds II and III.



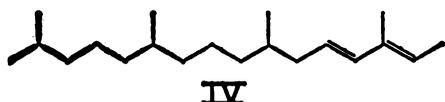
The ultraviolet spectra are those of conjugated dienes and both compounds yield upon ozonolysis the identical C₁₆ aldehyde. Thus II and III must be the two possible geometric isomers of

Table 1. Gas chromatography and spectra of phytadienes and derivatives. Gas chromatogram temperature 4 deg/min, 1.8 m by 0.3 cm (outside diameter) steel column; 3.5 percent RTV 502 (filter free) on Chromosorb G, acid washed, DCMS treated; 3.5 percent Carbowax C20M on same; w, weak.

Compound	Retention index		Spectra (maxima)	
	RTV502	C20M	Ultraviolet (m μ)	Infrared (cm ⁻¹)
I	1841	1919	224	3095,1630(w),1590,991,906,896
II	1863	1951	234	3095,3020,1630,1590,987,904,835(w)
III	1878	1979	228	3095,3020,1640,1600,989,893,850(w)
IV	1901	2004	232	3030,1640,1620,964,830
Phytane	1812	1786		
C ₁₆ aldehyde	1561	1810		
C ₁₆ aldehyde	1668	1931		
Me ester of C ₁₇ acid	1858	2118		

1,3-phytadiene with the *cis* structure being assigned to the lower-boiling compound II.

The infrared spectrum of compound IV shows no terminal unsaturation but rather a disubstituted ethylene (*trans*) structure conjugated with a trisubstituted ethylene. This is in agreement with the position of the ultraviolet absorption peak and the production upon ozonolysis of a C₁₅ aldehyde. Thus IV



is one of four possible geometric isomers of 2,4-phytadiene.

The biochemical source for the phytadienes in zooplankton has to be sought in the phytol of the phytoplankton diet of the animals. Phytol is a labile compound which is dehydrated by mild catalytic action of acids and bases to mixtures of phytadienes. Dehydration easily takes place on chromatographic adsorbents (alumina, silica gel). The zooplankton phytadienes are not laboratory artifacts from phytol present in the animals; their presence in crude zooplankton extracts or distillates which have not been in contact with chromatographic adsorbents can be shown by gas chromatography. A mixture of phytadienes very similar to that encountered in the zooplankton is formed by catalytic dehydration of phytol with oxalic acid (3). This might suggest their formation by acid catalysis in the digestive tract of the animals.

Pristane (1), the phytadienes, and the related mono- and polyolefins constitute a group of closely related compounds of graded chemical resistance to nonbiological and biological degradation. We intend to study the spread of these compounds from their common source through the marine biosphere and hydrosphere.

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Protein Synthesis Inhibition: Mechanism for the Production of Impaired Fat Absorption

Abstract. *Treatment of rats with puromycin and acetoxycycloheximide results in a defect in intestinal lipid transport. Under these conditions rats given corn oil accumulate triglyceride within the intestinal cells and fail to develop the normal postprandial hyperlipemia. The observed interference in lipid transport appears to be a consequence of impaired chylomicron formation.*

The absorption of dietary triglyceride from the intestine involves the formation of specific lipid particles, chylomicrons, which are secreted from the mucosal cells and appear predominantly in the lymphatic system (1).

The chylomicron is a complex structure consisting of a central core of triglyceride, additional lipid components (phospholipids, cholesterol), and an outer protein surface (2).

Although the protein moiety is very small (0.5 to 2 percent), this component is probably essential for the movement of lipid out of the mucosal cell, especially since lipoproteins are required for the transport of lipids in the systemic circulation. Support for such a concept was obtained from observations that a rare hereditary disorder in which there is a deficiency of β -lipoproteins is associated with a striking defect in the movement of lipid out of the intestinal mucosal cells (3). We have now shown that the inhibition of protein synthesis in the rat by puromycin and acetoxycycloheximide is associated with interference in the absorption and transport of lipid from the intestine.

Female Sprague-Dawley rats (180 to 200 g) were fasted for 24 hours and then injected intraperitoneally with puromycin (15 mg) (4) dissolved in buffered salt solution (0.04M phosphate buffer, pH 7.4 in 0.154M NaCl); the drug was administered in a series of hourly injections of 2.5 mg for 4 hours followed by five injections of 1 mg each. One hour after the fourth injection the animals were given 1.5 ml of corn oil by intubation, and killed 2, 4, and 6 hours later. Plasma triglycerides were determined at intervals (see 5) and multiple jejunal sections were examined with light and electron microscopes.

In the normal animal lipid droplets appear rapidly within intestinal villous epithelial cells after corn oil adminis-

tration. Four hours after such administration microscopy of intestinal biopsies shows most of the stainable lipid within villous and submucosal lymphatics with relatively little lipid remaining within epithelial cells; however, the lymphatics of the mucosa and submucosa show an abundance of stainable lipid droplets. By 6 hours lipid droplets in the mucosa are generally sparse, even within lymphatics.

In contrast, rats treated with puromycin show progressive accumulation of fat within the intestinal epithelial cells. Six hours after corn oil administration the villous epithelial cells are laden with lipid droplets of varying size (Fig. 1). The lipid is distributed rather uniformly throughout the cells and appears morphologically unremarkable except for the very large size of some particles, which suggests that a coalescence of retained droplets has occurred. In comparison with normal controls, relatively little lipid is seen in the villous lymphatics at any time after corn oil administration.

The accumulation of fat within the mucosal cells of the puromycin-treated rats is reflected by a failure of the plasma triglycerides to rise after a corn oil "load." Normal rats given 1.5 ml corn oil exhibit a progressive elevation of plasma triglycerides with the mean of the maximum values being 380 mg per 100 milliliters after 6 hours. In contrast, the means of the values for plasma triglycerides in the puromycin-treated animals barely rise above those of fasting animals (10 mg per 100 ml), and at 6 hours after administration of corn oil are only 37 mg per 100 ml, or 10 percent of those of the normal group (Fig. 2).

A similar defect in mucosal lipid

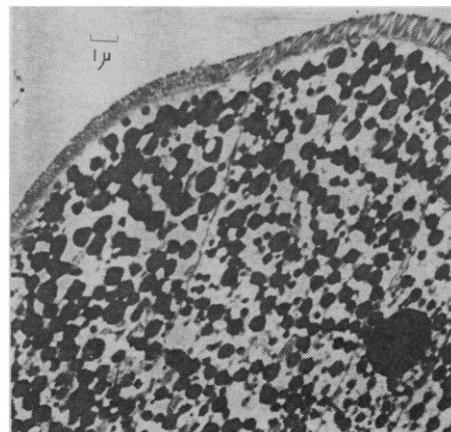


Fig. 1. Electron micrograph of small intestinal villous epithelial cells 6 hours after administration of corn oil (1.5 ml) in a rat treated with 15 mg of puromycin.